Journal of the Arkansas Academy of Science

Volume 55 Article 31

2001

Plasmid Analysis of Bacteria that Metabolize the Detergent Igepon

Russell O. Nordeen
University of Arkansas at Monticello

Thetsu Mon
University of Arkansas for Medical Sciences

David Gilmore
Arkansas State University

Plasmid Analysis of Bacteria that Metabolize the Detergent Igepon

Russell Nordeen*
School of Mathematical and Natural Sciences
University of Arkansas at Monticello
Monticello, AR 71656

Thetsu Mon
Department of Physiology
University of Arkansas for Medical Sciences
4301 West Markham
Little Rock, AR 72205

David Gilmore
Department of Biology
Arkansas State University
State University, AR 72467

* Corresponding Author

When humans go on long-term space missions, they will need basic life support supplies. The Advanced Life Support (ALS) program of the National Aeronautics and Space Administration (NASA) is currently testing bioregenerative life support systems to facilitate long-term space missions, including the colonization of Mars. In order to reduce resupply requirements to a space habitat, efficient recovery and recycling of resources from wastes is critical. Water comprises the largest mass in the waste of human-occupied habitats. Recycling of water is desirable since the costs associated with launch to low-orbit have been projected to be as high as $22,000/kg (Moses et al., 1989). It is estimated that each person on these long-term missions will require 27 kg per day thus making resupply costly and possibly curtailing scientific and other critical payloads.

Wastewater from showering, laundry, dishwashing, and other activities associated with personal hygiene contain low concentrations of contaminants. Soaps and detergents are the major organic contaminants. This wastewater is commonly know as "graywater". Graywater makes up the largest single waste stream comprising approximately 80% of total wastes (Gerba et al., 1995). The recycling of graywater to obtain potable water and water for personal hygiene use is essential for space habitats that are difficult to resupply. The reuse of graywater on Earth is also becoming a desirable goal as water demands are rapidly reducing water sources due to overuse of groundwater supplies and pollution.

NASA is developing plant production facilities as part of the ALS program for the purpose of water recycling and food production. Water recycling in these protocols would involve root associated microbial degradation of organic material, including detergents in graywater, and plant transpiration and condensation to purify the water. Igepon, a linear alkyl methyl taurate detergent currently in use on the International Space Station and a common ingredient of soaps and detergents, is actively degraded by bacteria in hydroponic systems (Bubenheim et al., 1997). The taxonomy and genetics of bacteria that degrade igepon are currently being investigated.

Plasmids are extrachromosomal DNA elements that replicate independently of chromosomal DNA and are often involved in the metabolism of xenobiotic compounds, such as detergents. This study investigated the presence of plasmids in seventeen strains of bacteria that degrade igepon that were isolated from a variety of environments.

Bacterial strains that metabolize igepon were isolated by David Gilmore from environments surrounding Arkansas State University by growth on a medium containing igepon as the sole carbon source (Table 1). This medium contains per liter 10 ml of a 100x salt solution containing sodium sulfate 0.74 g/L, magnesium chloride 0.12 g/L, potassium phosphate (monobasic) 0.09 g/L, calcium chloride 0.64 g/L, sodium bicarbonate 2.75 g/L, potassium chloride 0.91 g/L, sodium chloride 4.22 g/L, and ammonium chloride 100 g/L as the nitrogen source. The composition of this medium is based on the expected concentrations of these compounds in graywater. Igepon was added from a 50x solution (5% w/v) for a final concentration of 0.2% w/v. All chemicals were obtained from Sigma Chemical company (St. Louis, MO) with the exception of igepon TC-42, which was generously supplied by K. Wignarajah (Moffett Field, CA).

To prepare this medium, 1.5 g of agar (Difco Detroit,

Table 1. Igepon Metabolizing Bacterial Strains

Strain	Biolog Identification	Source
ASU 1	Ralstonia species	soil
ASU 2	*Delftia acidovorans*	soil
ASU 3	ND	soil
ASU 4	ND	soil
ASU 5	*Pseudomonas putida*	soil
ASU 6	*Stenotrophomonas maltophila*	water
ASU 7	ND	water
ASU 8	*Ochrobactrum anthropi*	compost
ASU 9	*Pseudomonas putida*	compost
ASU 10	ND	compost
ASU 11	*Stenotrophomonas maltophila*	sewage sludge
ASU 12	ND	sewage sludge
ASU 13	*Pseudomonas nitroreducens*	sewage sludge
ASU 14	*Ralstonia eutropha*	sewage sludge
ASU 15	ND	lettuce roots
ASU 16	*Ralstonia eutropha*	lettuce roots
ASU 17	ND	lettuce roots

MI) was added to distilled water. Following sterilization, 1 ml of sterilized 100x salt solution and 2 ml of filter sterilized 50x igepon solution were added after the medium had cooled to approximately 60° C. The medium was dispersed in 5 ml volumes into sterile test tubes and solidified as slants. Igepon degrading bacteria were subcultured on this medium every three to four weeks by incubating at 28° C for three days.

Bacteria were inoculated from the igepon slants into trypticase soy broth (TSB) medium (Difco) and grown overnight at 28° C until stationary phase (typically 18 to 22 hours depending on the strain). Cell densities were adjusted using a spectrophotometer set at 600 nm for plasmid procedures using Qiagen (Valencia, CA) plasmid DNA isolation columns as suggested by the manufacturer. Initially a "miniprep" alkaline sodium dodecyl sulfate (SDS) procedure was used (Hruby et al., 1990). Another plasmid isolation method used was a cleared lysate procedure that employed the detergent Triton X-100 for lysis of cells (Scott et al., 1981). The final procedure employed the use of Qiagen miniprep plasmid DNA isolation columns. The protocol of the manufacturer was used in these procedures. For gel electrophoresis of plasmid DNA, 1/5 volume of loading dye containing bromophenol blue as tracking dye (Sambrook et al., 1989) was added to approximately 1 μg of DNA sample in a 10 mM Tris base 1 mM EDTA (ethylenediaminetetracetic acid) buffer, pH 8.0, and loaded on a 0.7% agarose (BioRad Hercules, CA) gel. Gels were electrophoresed following standard procedures in a Tris Acetate buffer at 5 volts per cm.(Sambrook et al., 1989). Gels were stained in a 2 μg/ml solution of ethidium bromide for 10 min, destained in distilled water, and examined on a UV transilluminator for the presence of plasmid DNA. Photographs of gels were made with a Polaroid camera using 667 black and white film and a Tiffen yellow UV filter (Fisher Scientific Plano, TX). Plasmid DNA standards consisted of pBlu 5.5 kb (Carolina Biological Supply Burlington, NC) and pMO1896 approximately 57 kb, which is a Tn*5* loaded derivative of the IncP10 plasmid R91-5 in *Pseudomonas aeruginosa* strain PAO 11 (Nordeen and Holloway, 1990; Chandler and Krishnapillai, 1977). All chemicals used in plasmid DNA isolation procedures were obtained from Sigma chemical company.

Initial attempts to isolate plasmids using the mini-prep alkaline SDS method failed to show the presence of plasmids in any of the seventeen igepon strains examined in this study. During this preliminary phase of the research, one strain obtained from lettuce roots (ASU17) failed to grow on the igepon cultivation medium and was not further characterized. The mini-prep alkaline SDS method is designed for high copy number, low molecular weight plasmids such as pBlu. The pBlu plasmid was routinely isolated in high yields (3-4 μg/ml of culture) utilizing this method. These results suggested that the igepon strains did not contain low molecular weight, high copy number plasmids. Some bacteria are difficult to lyse due to differences in cell wall or cell membrance structure, so a cleared lysis method that works well with bacteria that are difficult to lyse with SDS was tried (Scott et al., 1981). This method is routinely used to isolate both low molecular weight, high copy number plasmids, as well as high molecular weight, low copy number plasmids from *Pseudomonas* species such as *P. aeruginosa.* This method also failed to indicate the presence of plasmid DNA in the sixteen igepon metabolizing strains examined. The final plasmid isolation procedure involved the use of commercial plasmid DNA isolation columns from Qiagen. These columns are essentially anion exchange columns that can be used to successfully elute plasmid DNA under high salt conditions. Two of the sixteen igepon metabolizing strains, ASU12 and ASU15, showed the presence of high molecular weight plasmids using this procedure. As an internal control, pBlu in *E. coli* HB101 and pMO1896 in *P. aeruginosa* strain PAO11 were included in each isolation procedure to determine the effectiveness of isolating low molecular weight, high copy number and high molecular weight, low copy number plasmids, respectively. These plasmids were routinely isolated using this procedure, which lends credibility to the Qiagen DNA isolation procedure. The plasmids from strains ASU12 and ASU15 were clearly larger than pMO1896, although the precise molecular weight has not been characterized. It is estimated that these plasmids are between 60 and 100 Kb in size based on comparisons with the migration of pMO1896; however, they may be larger (Fig. 1). There are two plasmids in strain ASU12 because a doublet is clearly resolved during prolonged electrophoresis with both plasmids larger than pMO1896 (Fig. 1).

Although we can not rule out the presence of other plasmids in strains ASU12 or ASU15 or in the other fourteen igepon metabolizing strains examined, we find this possibility remote due to the failure to isolate plasmids using a variety of isolation procedures and the ability to routinely isolate both low molecular weight (pBlu) and high molecular weight (pMO1896) plasmids with the Qiagen plasmid DNA isolation columns. The exact size of these plasmids will be determined by restriction enzyme digestion; however, an increase in the yield of these plasmids is necessary for these procedures. The role of these large plasmids in the degradation of igepon has not been determined yet. To establish an igepon metabolizing phenotype, these plasmids will be excised from low melting temperature agarose gels and transformed in restriction deficient *E. coli* strains that fail to metabolize igepon. Growth of these strains on the igepon cultivation medium will indicate the possible presence of genes involved in igepon metabolism and will be the subject of subsequent investigations.

ACKNOWLEDGMENTS.—We thank Kanapathipillai Wignarajah of the NASA Ames Research Center at Moffet

Fig. 1. Agarose gel electrophoresis of plasmid DNA isolated using Qiagen plasmid DNA isolation columns. Lane 1 ASU 15 Lane 2 pMO1896 (56.7 kb) Lane 3 ASU 12 Lane 4 pBlu (5.5 kb). Arrow to the left of Lane 1 indicates position of plasmid in strain ASU 15. Faint smeared bands in the middle of the gel are chromosomal DNA. Intense band at bottom of gel in Lane 4 is covalently closed circular form of pBlu, band above this is an open circular form of pBlu.

Field, CA, for his generous donation of igepon TC-42 for these studies. Pamela Bostian assisted in the initial stages of this research and was funded by a grant from the Arkansas Space Grant Consortium. This study was funded in part by a UAM academic research grant and grants from the Arkansas Space Grant Consortium. We are grateful to the School of Mathematical and Natural Sciences for providing research space and facilities to conduct these studies.

Literature Cited

Bubenheim, D., K. Wignarajah., W. Berry, and **T. Wydeven**. 1997. Phytotoxic effects of gray water due to surfactants. J. Amer. Soc. Hort. Sci. 122:792-796.

Chandler, P. M. and **V. Krishnapillai.** 1977. Phenotypic properties of R factors of *Pseudomonas aeruginosa*: R factors transferable only in *Pseudomonas aeruginosa.* Genet. Res. 23:251-257.

Gerba, C. P., T. M. Straub, J. B. Rose, M. M. Karpiscak, K. E. Foster, and **R. G. Brittain**. 1995. Water quality study of graywater treatment systems. Wat. Res. Bull. 31:109-116.

Hruby, D. E., J. M. Brinkley, H. C. Kand, R. P. Haugland, S. L. Young, and **M. H. Melner.** 1990. Mini-Prep in Ten Minutes. BioTecniques 8:172-173.

Moses, W. M., T. D. Rogers, H. Chowdhury, and **R. Cusick.** 1989. Performance characterization of water recovery and water quality from chemical/organic waste products. Paper 891509, 19th Intl. Soc. Conf. Environ. Systems. Soc. Automotive Eng. Int., Warrendale, PA.

Nordeen, R. O. and **B. W. Holloway.** 1990. Chromosome mapping in *Pseudomonas syringae* pv. *syringae* strain PS224. Jo. Gen. Micro. 136:1231-1239.

Sambrook, J., E. F. Fritsch, and **T. Maniatis.** 1989. Molecular cloning. A laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Scott, K. F., B. G. Rolfe, and **J. Shine.** 1981. Biological nitrogen fixation: Primary structure of the *Klebsiella pneumoniae nifH* and *nifD* genes. J. Mol. Appl. Gen. 1:71-81.

187